Morphological Anomalies in Fourth-Instar *Aedes aegypti* (Diptera: Culicidae) and a Case of Gynandromorphism in *Culex quinquefasciatus* (Diptera: Culicidae) in Yucatan, Mexico[1]

Carlos M. Baak-Baak, Julio C. Tzuc-Dzul, Rodrigo J. Orozco-Chulin, Wilbert A. Chi-Chim, Nohemi Cigarroa-Toledo[2], Rosa C. Cetina-Trejo, Lourdes G. Talavera-Aguilar, Nora A. Hernandez-Cuevas[3], and Julian E. Garcia-Rejon[4]

Laboratorio de Arbovirología. Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”. Universidad Autónoma de Yucatán. Mérida, Yucatán, México. C.P. 97225

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Abstract Two cases of morphological anomalies were documented in natural populations of mosquitoes collected in Yucatán, México. Of the fourth-instar *Aedes aegypti* (L.) collected, 3.81% (25 of 656) had anomalies that consisted of a chitinized bulb at the end of the siphon. The siphon also displays 2 pectens, 1 in the middle and 1 at the base of the chitinized bulb. The seta 1-S triple developed twice in the siphon, that is, in the middle and distally at the base of the chitinized bulb. Larvae display 2 combs in 2 separate curved rows: 1 in the eighth abdominal segment and 1 in the base of the siphon. The anal segment had double development; the first did not present a saddle and the ventral brushes. The other was less developed displaying a saddle, dorsal brushes, and ventral brushes. One *Culex quinquefasciatus* Say adult displayed a gynandromorph of the polar type, with the head of a female (filiform antennae, palps shorter than the proboscis), and an abdomen with male genitalia. Phenotypic anomalies in mosquitoes occur in nature, negatively affecting their reproductive success and reducing their vector competence.

Key Words development anomalies, mosquitoes, phenotypic anomalies

In nature, mosquitoes suffer from phenotypic anomalies (Barreto et al. 2008, De la Vega et al. 2020, Kronefeld et al. 2013, Roth 1948). Possible causes include genetic mechanisms such as binucleated eggs, recombination during mitosis, and generation by symbionts with deletion or destruction of the sex chromosome as another possibility (Narita et al. 2010). Furthermore, mosquito phenotype and physiology can alter due to heat stress, pollution, insecticide exposure, and nutrient restriction (Garcia-Rejon et al. 2018, Perez and Noriega 2014). The anomalies in mosquitoes (Diptera: Culicidae) have been described in terms of the structural anomalies of the larvae, which include an irregular development of setae on the saddle (Verna and Munstermann 2011). In adult mosquitoes, gynandromorphism (gyn: female, andro: male) has been reported

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[2] Laboratorio de Biología Celular. Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”. Universidad Autónoma de Yucatán. Mérida, Yucatán, México. C.P. 97000.

[3] Laboratorio de Parasitología, Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”. Universidad Autónoma de Yucatán. Mérida, Yucatán, México. C.P. 97225.

[4] Corresponding author (email: julian.garcia@correo.uady.mx).

(Ali and Rasheed 2008, Barreto et al. 2008, De la Vega et al. 2020, Mahmood and Bajwa 2006). It is a phenotypic abnormality in which mosquitoes present mixed morphological and structural traits in males and females (Hall 1988, Kronefeld et al. 2013). Gynandromorphy in mosquitoes is classified as polar, bilateral, and oblique. Polar gynandromorphs have a head of 1 sex and an abdomen of the other. The line of separation between the sexes follows the longitudinal plane in bilateral forms. In oblique gynandromorphism, the abdomen and one side of the thorax belong to one sex while the head belongs to the other (Barreto et al. 2008, De la Vega et al. 2020, Mahmood and Bajwa 2006, Roth 1948). Mosquito gynandromorphism is widespread, with 60 species and 12 genera documented. Gynandromorphism is observed most frequently in *Culex* and *Ochlerotatus* mosquitoes, documented in 14 and 12 species, respectively. This may be a strategy of their high species richness and frequency of observation, given their medical importance (De la Vega et al. 2020, Kronefeld et al. 2013, Mejias-Ortiz et al. 2019). Furthermore, it has been reported in several countries in mosquitoes caught in the wild or reared in laboratories (Ali and Rasheed 2008, Barreto et al. 2008, De la Vega et al. 2020, Mejias-Ortiz et al. 2019). In this work, we present 2 cases of mosquitoes with morphological anomalies.

Materials and Methods

Mosquito collection. In June 2022, larvae of *Ae. aegypti* (n = 656), *Aedes albopictus* (Skuse) (n = 56), and *Culex coronator* (Dyar & Knab) (n = 38) were collected at a residence in the rural community of Cuzama, Yucatan (20° 44’ 18.499” N, 89° 19’ 4.987” W). The immatures of the 3 species were extracted from the same plastic container which was used to store drinking water and had a capacity of approximately 210 L. At the time of collection, the container was filled one-third with water (approx. 70 L). The container was found under shade with abundant organic matter, mainly leaves of the *Brosimum alicastrum* Swartz tree (Rosales: Moraceae).

Between January and February 2023, adult mosquito collections were conducted weekly in the zoological facility (20° 58’ 9.13’’ N, 89° 38’ 24.609’’ W) in the city of Merida, Yucatan. A backpack-mounted aspirator (Prokopack Aspirator®, model 1419, John W. Hock Co., Gainesville, FL) was used to collect adult mosquitoes of *Cx. quinquefasciatus* Say, *Cx. nigripalpus* Theobald, and *Ae. aegypti*.

Taxonomic identification of mosquitoes. The mosquitoes were transported to the Arbovirology Laboratory of the Dr. Hideyo Noguchi Regional Research Center of the Autonomous University of Yucatan (CIR-UADY), where they were identified to the species level following dichotomous keys Carpenter and La Casse (1974) and Rueda (2004).

Results

In total, 3.81% (25 out of 656) of the fourth instars of *Ae. aegypti* collected had an irregular development, mainly in the eighth and tenth abdominal segments (Fig. 1B–D). Ten larvae displayed the comb of the eighth abdominal segment with 5 to 8 comb scales in a single curved row. Comb scales are typical of *Ae. aegypti*, with a strong median spine and 2 smaller spines on the sides. The distal part of the siphon displays a chitinized bulb with 9 to 15 irregular pecten spines. The siphon displayed 2 pectens, that is, one in the middle and one at the base of the chitinized bulb. Seven to 10 pecten

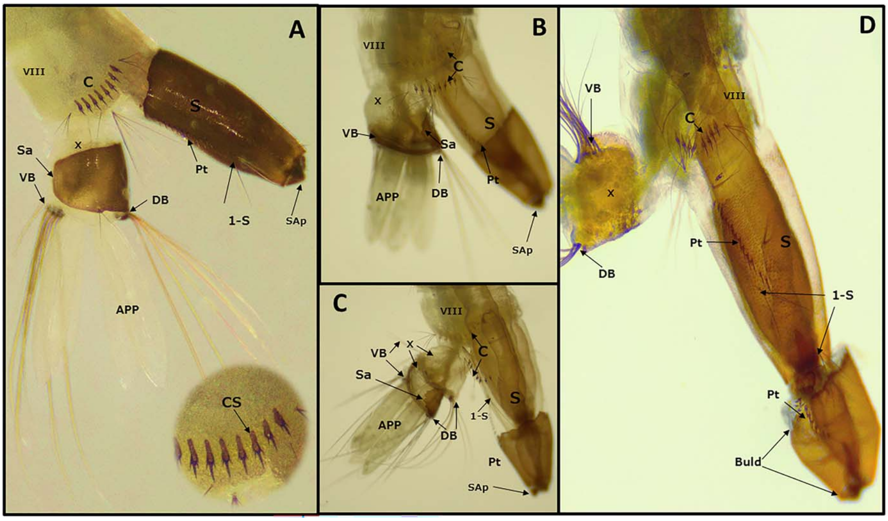

Fig. 1. The fourth-instar larva of *Ae. aegypti*: normal (A) versus abnormal (B, C, D). (B) larvae display 2 combs (C) in 2 separate curve rows: one in the eighth (VIII) abdominal segment and one in the base of the siphon (S); (C) the anal segment (X) had double development; the first did not present a saddle (Sa), and the ventral brushes (VB). The other was less developed; it displays a saddle (Sa), dorsal brushes (DB), ventral brushes (VB), and anal papillae (APP); and (D) comb (C) of the eighth (VIII) abdominal segment with 5 to 8 comb scales (CS) in a single curved row. The distal part of the siphon (S) displays a chitinized bulb. The siphon displays 2 pectens (Pt), one in the middle and one at the base of the chitinized bulb. The seta 1-S triple (1-S) developed twice in the siphon: in the middle and distally at the base of the chitinized bulb.

spines were observed at the base of the chitinized bulb (Fig. 1D). The seta 1-S triple (1-S) developed twice in the siphon, i.e., in the middle and distally at the base of the chitinized bulb (Fig. 1D). Fifteen larvae possessed 2 combs in 2 separate curved rows, one on the eighth abdominal segment and one on the base of the siphon (Fig. 1B). The anal segment (X) had double development. The first did not present a saddle and the ventral brushes. The other was less developed displaying a saddle, dorsal brushes, and ventral brushes. The saddle on the anal segment did not reach the usual size and presented an irregular shape (Fig. 1C). It should be noted that the larvae with anomalies had mobility that was typical of normal larvae.

The normal fourth instars of *Ae. aegypti* present the following combination of characteristics: comb of the eighth segment with 7 to 12 scales in a single curved row; individual scales are thorn-shaped, with a strong median spine and several shorter, stout lateral spines. A pecten of about 10 to 19 evenly spaced teeth reaches the middle of the siphon; a siphonal tuft is 2- to 5-branched (usually triple), inserted beyond the pecten. The anal segment has a saddle that extends about seven-eighths of the way down the sides, and the lateral hair can be single or double and is about as long as the

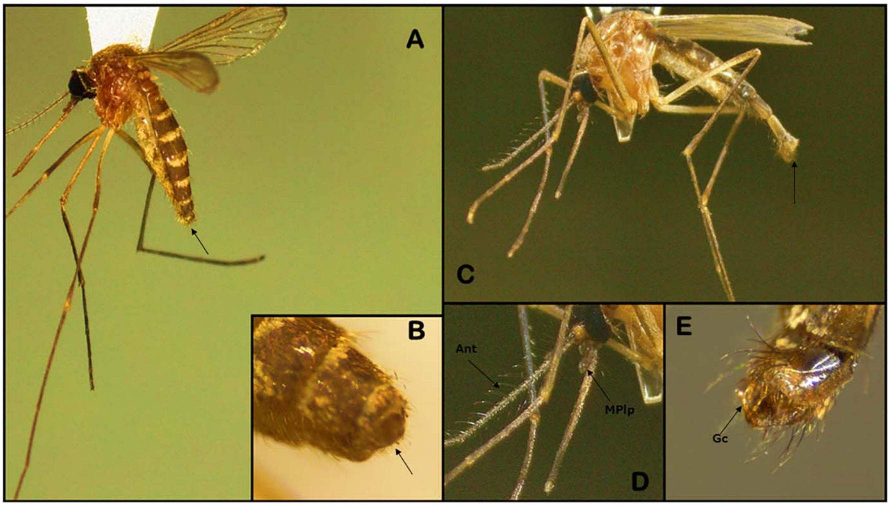

Fig. 2. Normal female *Cx. quinquefasciatus* (A, B) versus specimens with polar gynandromorphy (C, D, E). (B) the terminal segment of the abdomen of a female; (C) specimen with polar gynandromorphy showing female head and male genitalia (arrow); (D) head with filiform or less plumose antennae (Ant), maxillary palpus (MPlp) shorter than the proboscis; and (E) male genitalia with gonocoxite (Gc).

saddle. The dorsal brush has a long lower caudal hair and a 2- to 4-branched upper caudal tuft on each side, and the ventral brush has about 7 to 10 long double hairs that are only in the barred area. The gills are about 3 times as long as the saddle and are broad and bluntly rounded at the tip (Fig. 1A).

A total of 1,089 adult mosquitoes were collected from the zoo in Merida, Yucatan. The preponderance of these were *Cx. quinquefasciatus* (658 males; 346 females) followed by *Ae. aegypti* (41 males; 21 females) and *Cx. nigripalpus* (13 males; 10 females). A single adult *Cx. quinquefasciatus* exhibited the polar type of gynandromorphism having the head of a female (filiform antennae, palps shorter than the proboscis) and an abdomen with male genitalia (Fig. 2C–E). The mosquito had no blood in its abdomen.

Discussion

There are few reported cases of morphologic anomalies in *Ae. aegypti*, most of which are slight. We have not found other reports of the deformities of similar severity to those observed in our samples. A slight anomaly consisting of a reduction in the length of the lateral saddle seta was previously reported by Verna and Munstermann (2011). Perez and Noriega (2014) also reported that *Ae. aegypti* larvae reared under copper (Cu^{2+}) environmental stress underwent phenotypic and physiological alterations. The tissue of the mosquito larvae and adults was less robust, pale, and translucent in appearance. In addition, adults had reduced body mass, fewer reserves of neutral lipids at emergence, and a reduced tolerance to starvation. The phenotypic abnormality of

Ae. aegypti in the current study, however, was severe, and we are unsure if it was due to a compound or a coding error in the mosquito genome.

Roth (1947) reported the first occurrences of gynandromorphism in *Cx. quinquefasciatus* was reported from the United States. An insect showed oblique gynandromorphism, for example, appendages of one side of the body (antenna, palp, foreleg, and midtarsi) were of the male type, while the genitalia and spermathecae were of the female type. Another *Cx. quinquefasciatus* presented polar gynandromorphism, with antennae and palps characteristic of males and genitalia characteristic of females. In Pakistan, *Cx. quinquefasciatus* with polar gynandromorphism was also identified. The head and thorax had female characteristics, while the abdomen had well-developed male genitalia (Ali and Rasheed 2008). The specimen we identified coincides with the one reported in Pakistan. The doublesex gene controls the somatic sexual differentiation in some mosquitoes. Among the genes, it is known that Agdsx, Nix, and dsx can control sex determination in *Anopheles gambiae* Giles, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively. Disruption of the genes can promote the development of male genitalia in female mosquitoes (or vice versa), thus causing development of a gynandromorph (Hall et al. 2015, Kyrou et al. 2018, Price et al. 2015).

Gynandromorphism impacts the reproductive success of the individual. Because of its rarity it likely does not impact overall population-level reproductive success. Polar mosquitoes with female anterior portions respond to mating stimuli, but the presence of male genitalia reduces the probability of copulation. Likewise, if the females take blood from the hosts, they are not be able to oviposit the eggs.

In conclusion, *Ae. aegypti* presented severe external anomalies, mainly in segments VIII to X. However, we observed the movements of a normal larvae in the water. Unfortunately, we did not follow the development and growth, and we could not observe how it impacts the pupae and adult or if there is mortality between molts. In the future, researchers should investigate how the leaves and seeds of *B. alicastrum* affect the growth of young *Ae. aegypti* and investigate possible causes that were not investigated in this study, like heavy metals in the breeding site. It is also important to look for changed enzymes and resistance genes that are connected to the main insecticides to which the mosquito populations are exposed, such as carbamates, organophosphates, and pyrethroids. Currently, there are no experimental molecular studies aimed at understanding the development of gynandromorphism. The dsx gene of *Cx. quinquefasciatus* with and without gynandromorphism should also be studied further.

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The authors declare no competing interests relevant to the contents of this article.

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